Lea Harrington: Short Bio and Affiliation

Lea Harrington is interested in the dosage-sensitive regulation of telomere homeostasis. Her lab uses genetic model systems to probe the consequences of telomere instability in aging, cancer, and disease. A current focus is the genetic and epigenetic vulnerabilities that affect cancer and stem cell fate when telomeres become eroded.

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Duncan Baird: Short Bio and Affiliation

Duncan Baird is professor of Cancer and Genetics, at Cardiff University in the UK. His group focuses on understanding the mechanistic basis of telomere erosion, instability and fusion, and how these impact on the evolution of the cancer genome and tumour progression.

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Introduction

Tentative title: The instability of the cancer genome: it starts at the end

Cancer is a complex disease that affects many cell types and tissues. Even within one tissue, there are numerous different tumour types that can occur. For example, ovarian cancers can occur as epithelial-derived tumours, germ cell tumours, stromal tumours, sarcoma, and so-called Krukenberg tumours, each with several subtypes leading to at least 16 distinct tumour classifications (https://www.cancercenter.com/cancer-types/ovarian-cancer/types).

Another layer of complexity is the cancer genome itself. Even within one cancer subtype, the genomic variation within the tumour may be incredibly complex. For example, within one type of breast cancer, Ductal Carcinoma In Situ (DCIS), there is considerable genomic heterogeneity between patients, but also within the tumour itself [1].

Indeed, a recent pan-cancer analysis of whole genomes revealed a staggering complexity between cancer genomes [2]. One of the lessons learned from these studies is that mutations within the cancers serve different roles. Some mutations are acquired during tumour progression but do not necessarily facilitate tumour progression (termed a passenger mutation). Other mutations that are considered to confer a fitness advantage are known as driver mutations. In cancers, there may be as many as 4-5 driver mutations on average within a cancer genome, but in up to 5% of cancers no evident driver mutations are found [2]. The complex temporal and functional relationship between driver and passenger mutations is only beginning to be explored.

There is also a huge degree of chromosomal rearrangement and instability during tumour progression. The cancer genome is shaped and evolved continuously by mutations that affect the entire landscape of genome stability; this state can include loss of DNA replication fidelity and repair, as in the mismatch repair genes; states that lead to loss in epigenetic regulation of chromatin, as in the loss of IDH and other chromatin remodeling functions in glioblastoma; and in mutations that affect stability of telomeres. As one example

of the latter, mutations in the telomerase reverse transcriptase, the enzyme that replenishes telomeres, is now one of the most prevalent mutations in cancer [2](see below).

This journal special issue is dedicated to cancer genomics, with a focus on how telomere instability contributes to the complexity of phenotypes and genome rearrangements found in cancer. The contributing authors, all leaders in their respective fields, have provided important and topical summaries that delve into the mechanisms by which loss of telomere integrity shapes the cancer genome. To gain an appreciation of how model organism research has underpinned much of our understanding of how telomeres drive genome instability, Erin Henninger and Teresa Teixeira provide a broad-ranging review of how yeast models have been used to molecularly dissect telomere-driven mutational processes, including DNA repair and resection, replication stress and chromatin. They also discuss the role that telomeres play in driving genomic evolution. The sub-telomeric regions of eukaryotic chromosomes are highly recombinogenic and this creates extensive structural and sequence variation; these regions of the genome often contain diverse gene families that can facilitate adaptation to ecologic niches, for example, the virulence genes in parasitic eukaryotes, olfactory receptors in mammals and genes involved in disaccharide metabolism in yeast. They consider the intriguing "adaptive telomere failure hypothesis", whereby subtelomeric recombination in response to environmental stress may confer selective advantages. They take this notion further by considering that subtelomeric sequence evolution could also be driven by episodic telomere erosion, dysfunction and fusion driving large-scale, potentially adaptive, genome changes. Model organisms have contributed hugely to our understanding of telomere biology, and so too have advances in analytical technology. Veronica Cherdyntseva and Sarantis Gagos provide an overview of the ongoing contribution that sophisticated molecular cytogenetic technologies (see cover image) have made in illuminating the role of telomeres in genome integrity. They focus on the contribution these technologies have made to our understanding of how telomeric replication defects can trigger RAD52-dependent, and RAD52-independent, break-induced replication (BIR) and telomere elongation in cells undergoing the alternative lengthening of telomeres (ALT) phenotype in the absence of telomerase.

Protein complexes that play important roles in protecting and replicating telomeres are also a source of mutation in cancer, and the role of variants in one of these proteins in cancer, POT1, is the subject discussed by Yi Gong, Amanda Stock and Yie Liu. They review the burgeoning evidence that mutations in shelterin subunits, such as POT1, TPP1, and RAP1, lead to hyper-elongated telomeres and an elevated risk of cancer, and discuss the potential mechanisms by which these mutations may confer a selective advantage to the cancer genome. They review how germline mutations in POT1 lead to long and fragile telomeres, and are associated with familial melanoma. Finally, they discuss how factors that cooperate with shelterin to replicate telomeres, such as the CST complex (Cdc13-Stn1-Ten1), may also play a role in the instability of long telomeres. Alessandro Cicconi and Sandy Chang discuss the role of shelterin at both telomeres and the replisome. They review the factors that dictate the choice of DNA repair pathway that occurs at a de-protected telomere compared with double-strand breaks throughout in the genome. Telomere replication also plays a role in mediating the repair of dysfunctional telomeres, and they discuss recent evidence that shelterin, the helicase RTEL1, and components of the replisome cooperate to ameliorate replication stress at telomeres.

One of the emerging themes in cancer genome evolution is that telomere instability may be an early event. This observation is true both for cancers that reactivate telomerase, and those that invoke telomere recombination-based mechanisms. For example, telomerase upregulation is a key feature of many cancers. One mechanism that affects *TERT* expression is methylation of its promoter. Donghyun Lee, Martin Komosa, Nuno Nunes, and Uri Tabori review the identification of a region upstream of *TERT*, called the *TERT* Hypermethylated Oncological Region (THOR) whose DNA hypermethylation, paradoxically, leads to *TERT* upregulation in several cancer types. They discuss the potential mechanisms by which this epigenetic regulation occurs, and its implications for cancer diagnosis, prognosis, and therapy. Another mechanism of telomerase upregulation in cancer is via mutations in the *TERT* promoter that lead to TERT

upregulation, as discussed by Franziska Lorbeer and Dirk Hockemeyer. Indeed, as Lorbeer and Hockemeyer discuss, promoter mutations in *TERT* are now appreciated to be a prominent driver mutation that occurs in many different types of cancers [2]. Interestingly, these *TERT* promoter mutations do not necessarily lead to telomere elongation, and the potential implications of this finding are manifold. On the flip side, telomerase-negative cancers also have key defining features that give them a survival advantage; Alexander Sobinoff and Hilda Pickett review these alternative lengthening of telomeres (ALT)-based mechanisms, and they underscore that these events are more prevalent and complex than originally supposed. For example, there are at least two types of break-induced replication mechanisms that drive ALT, and how these recombination intermediates are processed also plays a key role in the outcome of ALT (e.g. dissolution versus resolution). Eloïse Claude and Anabelle Decottignies delve into other intriguing aspects of ALT, including instances in which telomerase and ALT may co-exist within tumours. Even more surprising, there is emerging but strong evidence that tumours may lack both telomere maintenance mechanisms (TMM) altogether. They posit that these tumours may represent a distinct class of cancer with a more favorable prognostic outcome.

Indeed, the genomic rearrangements that help cells cope with insufficient telomere maintenance can be drivers of genetic instability and disease in themselves. Cecile Herate and Laure Sabatier provide a discussion about the consequences of telomere dysfunction in cells undergoing a telomere-driven crisis. They consider a 'butterfly' effect whereby telomere dysfunction leads to chromosomal fusion and the initiation of anaphase bridging and breakage-fusion-bridge cycles. These events, in turn, result in a spreading of loss of heterozygosity in telomeric regions that reveal accumulated recessive mutations and that could further exacerbate telomere dysfunction and genomic mutation. Telomere fusion between sister chromatids, or heterologous chromosomes, leads to the formation of dicentric chromosomes. Susanna Stroik and Eric Hendrickson review the mechanisms of telomere dysfunction that leads to telomere fusion and how dicentric chromosome are resolved at anaphase. They consider how telomeric replication fork stalling can arise from the loss of replication proteins, RNA:DNA hybrids, free radicals, and aberrant DNA structures, ultimately leading to telomere dysfunction and fusion. The resolution of dicentric chromatin bridges is considered to occur by physical shearing or via the action of the cytosolic exonuclease TREX1. However, in contrast, the RPA coated ultrafine bridges are protected from physical rupture by the binding of the Plk-Interacting Checkpoint Helicase, that also recruits the Bloom Syndrome helicase (BLM). Subsequent unwinding and nucleolytic processing renders UFBs single stranded, but how single-stranded UFBs are ultimately resolved has not been established. Sally Dewhurst examined the wider genomic implications of telomere dysfunction during telomere-driven crisis. Whole genome sequencing of cancer cells has revealed scars of telomere driven mutation, including the phenomenon of chromothripsis whereby 100-1000s of breakpoints are clustered into localised regions. Chromothripsis can be initiated by telomere dysfunction and may arise from replicative and/or nucleolytic processing of chromatin bridges or micronuclei. The link between genomic catastrophe and innate immune signalling via the cGAS/STING pathway was also considered. Another consequence of telomere-driven instability is how the repair of broken ends through breakage-fusion-bridge cycles and chromothripsis further drive genomic instability. In this issue, Alessandra Brambati, Raymond Barry and Agnel Sfeir shed light on how the repair itself can be mutagenic, through error-prone polymerase activity such as DNA polymerase theta. Pol theta carries out microhomology-mediated end joining at de-protected telomeres and elsewhere in the genome. They discuss the various roles of this polymerase in normal cells and cancer, and implications of the recent discovery that its function may contribute to the survival of HR-deficient cancer cells. Finally, Ragini Bhargava, Matthias Fischer and Roderick O'Sullivan extend the discussion of genomic catastrophe to consider how the subsequent complex structural rearrangements, such as breakage-fusion-bridge cycles, chromothripsis, and kataegis can promote telomere maintenance, via telomerase or ALT, and facilitate the ability of cells to escape crisis and to drive cancer.

Telomere shortening, dysfunction and fusion has clear clinical implications. Kristen Schratz and Mary Armanios discuss how the short telomeres observed in patients with inherited telomere maintenance mutations have a propensity to bone marrow failure and the development of myeloid leukemias. Interestingly, whilst young patients with short telomeres typically develop aplastic anemia, older patients will typically develop myelodysplasia and/or acute myeloid leukemia. They consider that this difference may relate to the progressive decrease in haematological oligoclonality as a function of age, that may be exacerbated in the context of short telomere syndromes due to haematopoietic stem cell drop out and clonal selection. This scenario has implications for androgen therapy used to treat these conditions. Whilst the short telomere syndromes present an extreme of telomeric phenotypes in the human population, telomere length in the normal population displays considerable heterogeneity. This variability can be partially accounted for by genomic variants in genes known to be involved in telomeric regulation including TERT and RTEL1, as well as other genes, including ACYP2, PXK and DCAF4, that have no obvious role in telomere biology; however in total this variation accounts for just 4% of the total variation in telomere length. Christopher Nelson and Veryan Codd review this area and how our current understanding of how GWAS and Mendelian randomisation studies have established longer telomere length as a causal factor for cancer risk. The majority of genomic variants that affect telomere length have been identified in European populations, and it is clear that further studies encompassing a broader diversity of the human population are required to establish if polygenic scores for telomere length could be used for cancer risk prediction. While constitutive telomere length variation can influence the risk of developing cancer, it is also clear that the telomere length of tumour cells can directly influence genome instability and clonal evolution. Chris Pepper, Kevin Norris and Christopher Fegan discuss how high-resolution telomere length analysis has been used as to determine both prognosis and response to treatment across of a range of tumor types.

Human disease can also arise not just from telomere loss or gain, but loss of epigenetic mechanisms that regulate telomeres, as discussed by Shir Toubiana and Sara Selig. For example, perturbations in the methylation of sub-telomeric DNA leads to aberrant regulation of telomere transcripts, TERRA. In ALT, hypomethylated sub-telomeric DNA leads to upregulation of TERRA which in turn drives telomere recombination via an increase in DNA-RNA hybrids. Dysregulation of subtelomeric DNA methylation is also found in human diseases associated with mutations in the de novo DNA methyltransferase Dnmt3b, such as the immunodeficiency syndrome ICF1.

In preparing this issue, we aimed to cover the wealth of data that shows how telomere dysfunction can drive genomic instability, clonal evolution and malignant progression. A full understanding of the underlying mechanisms of telomere dysfunction and fusion may yield therapeutic targets. For example, it will be intriguing to examine whether tumours exhibiting telomere dysfunction are sensitive to agents targeted to the DNA damage response. The application of telomere analysis in the clinic is already established for the relatively rare short telomere syndromes, and telomere analysis may become more widespread with the extension of its use as a prognostic and predictive marker for cancer. From the original observations of Hermann Muller and Barbara McClintock in the 1930's on the consequences of telomeric dysfunction, to our ability to detect the genomic scars with the molecular cytogenetics and whole genome sequencing of the modern era, it clear that the telomeres are fundamental to genomic integrity and a broad range of human diseases.

References

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